

SYMPOSIUM ON MICROBIAL AMINO ACID METABOLISM¹

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A biochemical relationship between the structurally similar branched chain amino acids, valine and isoleucine, was shown by Bonner, Tatum, and Beadle (1), who isolated a single mutant of *neurospora* which required both amino acids for growth. Tatum and Adelberg (2) isolated two α - β , dihydroxy acids having the carbon chains of valine and isoleucine from the corresponding *neurospora* mutants requiring these amino acids. Finding that C¹⁴-labeled acetic acid was incorporated into these substances, they suggested that both amino acids arise from acetic acid through some common intermediate.

In the experiments on valine, leucine, and isoleucine biosynthesis discussed by Murray Strassman, yeast was grown on glucose in the presence of C¹⁴-labeled substances, and valine, isoleucine, and leucine were isolated from the cell material and degraded chemically. Though acetate was not readily incorporated, all three lactate carbons were highly incorporated into specific positions of valine (3). The carboxyl carbon of lactate appeared exclusively in the carboxyl, the α -carbon almost completely, and equally in the α - and β -carbons, and the methyl carbon of lactate into the methyl carbons of valine. The following mechanism was proposed: (a) oxidation of lactate to pyruvate; (b) decarboxylation of pyruvate to acetaldehyde; (c) condensation of pyruvate and acetaldehyde to yield acetolactic acid; and (d) a molecular rearrangement, involving migration of a methyl carbon from carbon 2 of the pyruvate moiety to carbon 1 of the acetaldehyde moiety of acetolactate, to yield ultimately, the keto analog of valine. It seemed evident that the biosynthesis of

isoleucine might also proceed by an analogous series of reactions, involving a condensation of acetaldehyde with α -ketobutyric acid instead of pyruvic acid, to yield a homolog of acetolactic acid, α -aceto- α -hydroxybutyric acid. Migration of the ethyl group in this case would produce the carbon skeleton of isoleucine. The distribution of C¹⁴ from labeled acetates and lactates in isoleucine confirmed this suggestion (4). Adelberg (5) grew *neurospora* on labeled threonine and observed a distribution of C¹⁴ in isoleucine in agreement with the above proposal. However, he proposed a somewhat different mechanism, involving an aldol rather than a ketol condensation.

Relationships between the synthetic pathways of the homologous carbon chains of valine and leucine was first suggested by Abelson (6) who found, by means of isotopic competition experiments, that valine, its keto analog, and acetate are all precursors of leucine. He proposed that keto valine condensed with acetate to yield an intermediate which by decarboxylation yielded the carbon skeleton of leucine. Confirmatory evidence for such a mechanism was obtained by Strassman *et al.* (7). It was found that the carboxyl and methyl carbons of acetate were incorporated almost exclusively into the respective carboxyl and α -carbons of leucine; and the patterns of labeling in the isobutyl portions of leucine and of valine were identical, indicating that these portions of the two molecules have a common origin. It is suggested that the reactions involved may be analogous to those of the citric acid cycle, and that such a process may constitute a general mechanism for the conversion of an α -keto acid to its next higher homolog.

In a discussion of the microbial cleavage of heterocyclic rings, Jesse C. Rabinowitz made the following points: The bacterial degradation of the purine molecule involves the cleavage of its two heterocyclic constituents—the pyrimidine and imidazole ring nuclei. Certain striking similarities between this process and those involved in the degradation of the pyrimidine derivative, orotic acid, and the imidazole derivative, histidine, have been found.

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The degradation of the purine, xanthine, has been studied with extracts of *Clostridium cylindrosporum*, an organism isolated from soil by Barker and Beck (8) on a medium enriched with uric acid. The extracts first attack the pyrimidine moiety of xanthine to form 4-ureido-5-imidazole-carboxylic acid in a reaction which appears to be hydrolytic in nature (9). This substance is next converted to 4-amino-5-imidazolecarboxylic acid. This reaction requires a reducing agent and Mn^{++} or Fe^{++} . Similar reactions were encountered by Lieberman and Kornberg in studies on the metabolism of orotic acid with extracts of *Zymobacterium oroticum*. The pyrimidine ring is first reduced to dihydroorotic acid in a reaction with (DPNH) (10), and is then split hydrolytically to yield ureidosuccinic acid (11). This product is converted to aspartic acid, ammonia, and carbon dioxide, in a reaction requiring a reducing agent and Mn^{++} or Fe^{++} . The bacterial metabolism of the ureido group of citrulline has been shown to involve a reversible phosphorolytic cleavage (12-15) in which carbamyl phosphate and finally adenosine triphosphate (ATP) are formed (16). Although the phosphorolysis of ureidosuccinic acid has not been demonstrated in *Z. oroticum*, the reverse reaction in which carbamyl phosphate reacts with aspartic acid has been demonstrated by Jones *et al.* (16) with extracts of *Streptococcus faecalis*. The cleavage of the ureido group by extracts of *C. cylindrosporum* does not appear to be phosphorolytic.

With respect to cleavage of the imidazole ring—extracts of *C. cylindrosporum* decarboxylate 4-amino-5-imidazolecarboxylic acid to 4-amino-imidazole which is then, in unfractionated extracts, converted to formiminoglycine and ammonia. The last step requires a reducing agent and Fe^{++} . Although the mechanism of this reaction is not known, with partially purified extracts a product is formed from 4-aminoimidazole which can be differentiated from formiminoglycine. A similar series of reactions may be involved in the degradation of histidine by extracts of *Pseudomonas fluorescens* as demonstrated by Tabor and Mehler (17). Histidine is first converted to urocanic acid. The imidazole ring is then split to yield formiminoglutamic acid through the action of urocanase. The mechanism of the reaction is not known.

With extracts of *C. cylindrosporum*, formiminoglycine is converted to glycine, formic acid, and ammonia. Formylglycine is not an intermediate

in this process. After treatment of the extracts with "dowex"-1-chloride, their activity becomes dependent on the addition of orthophosphate, (ADP), and a folic acid derivative. With a partially purified fraction, it has been possible to demonstrate the following reaction: Formiminoglycine + P_i + ADP \rightarrow glycine + formic acid + ammonia + ATP. This reaction, therefore, could furnish a part of the energy required for the growth of the organism.

Jay V. Beck and Richard D. Sager reviewed their experiments on the hydroxymethyl and formimino group transfer reactions in the serine-glycine interconversion as follows: Degradation of purines by *Clostridium acidi-urici* leads, under certain conditions, to the accumulation of glycine and formate. Since the normal end products are acetate, ammonia, and CO_2 , it has been suggested that the catabolic sequence is through serine, pyruvate, and thence to the end products. Tracer studies gave results in agreement with the glycine to serine conversion. Attempts to convert glycine to pyruvate (via serine) were unsuccessful until formaldehyde was used instead of formate, whereupon rapid and complete conversion took place. Further study showed that tetrahydrofolic acid (THFA), prepared by catalytic reduction of pteroylglutamic acid (PGA) was essential for the conversion reaction.

Formiminoglycine (FIG) accumulates in stoichiometric quantities in the degradation of xanthine by extracts of dried cells or purified cell-free extracts of *C. acidi-urici* and *C. cylindrosporum*, although it is found in only small amounts following the use of intact cells or fresh extracts of intact cells. The degradation of FIG to ammonia, CO_2 , and glycine is also dependent on THFA. The activity of aged or dowex-treated extracts is restored completely by the addition of THFA.

Transfer of the formimino group from FIG to labeled glycine has been shown to be catalyzed by cell-free extracts of *C. acidi-urici* and to be dependent on THFA. This suggests that the active form of folic acid is formiminotetrahydrofolic acid (I) and that the reduction of formate to the aldehyde necessary for formation of serine may result from a reductive deamination of I.

Additional studies have shown that the slow direct oxidation of glycine which occurs in the presence of whole cells or extracts of *C. acidi-urici* is also dependent on THFA.

The following reactions are thus shown to be

THFA dependent: (a) formaldehyde + glycine → pyruvate (serine); (b) transfer of formimino group; (c) degradation of formimino glycine; and (d) oxidation of glycine.

Dr. Esmond E. Snell, in discussing the correlation of enzymatic events in amino acid synthesis with growth studies, stated that the objective of enzymatic study is to develop an explanation of growth phenomena in terms of simple chemical events. This correlation is, however, rarely made. In the lactic acid bacteria such a correlation of growth requirements and enzymatic events can be particularly exemplified.

The sparing effect of purine bases, thymine, and serine on the folic acid requirement of *Lactobacillus casei* and *Streptococcus faecalis* grown in "complete" media (18-20) led to the prediction that folic acid plays a role in the metabolism of these amino acids and that folinic acid functions in 1 carbon unit metabolism (21, 22).

A relationship of purine metabolism to histidine biosynthesis can be seen from the following observations: (a) in a folic acid free medium, histidine spares the purine requirement for *L. casei* (23); (b) although in yeast and lactic acid bacteria the imidazole ring of purine is not transferred to histidine (24-26), carbon 2 of guanine is transferred to position 2 of histidine (lactic acid bacteria) (26); (c) in the same organism formate also is transferred to histidine (24-26); (d) for lactic acid bacteria carbon 2 of purine bases is incorporated efficiently into histidine under circumstances that do not permit utilization of formate. This transfer represents a metabolic transformation of purine bases not accounted for by the preceding papers dealing with purine metabolism.

The role of vitamin B₆ in D-amino acid biosynthesis was revealed by the observation that: (a) in a complete medium, D-alanine replaces vitamin B₆ for the growth of *Streptococcus faecalis* (27); (b) D-alanine grown cells were essentially devoid of vitamin B₆ whereas vitamin B₆-grown cells (D-alanine free medium) contained D-alanine (27, 28). The interpretation of these data that vitamin B₆ functions in D-alanine synthesis has been verified by the finding of a pyridoxal phosphate-requiring alanine racemase in *S. faecalis* (29). D-Alanine remains as alanine in the cell, but is conjugated rather than free (30). It is confined mainly to the cell wall and is very low or absent in the intracellular protein. It is also

present in an unidentified extractive which may be an intermediate in cell wall formation.

Only a restricted number of other amino acids are present in the cell wall. D-Glutamate is present in high concentration (31). The route for its formation has been postulated to involve either the alanine racemase coupled with a D-alanine-D-glutamate transaminase (32), or a glutamate racemase (33, 34).

These examples illustrate a few ways in which observed interrelationships among nutrients in organisms with complex nutritional requirements can indicate the general nature of underlying enzymatic events within the cell, and permit an understanding of growth phenomena in terms of individual enzymatic conversions.

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